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Population collapse of *Lumbricus terrestris* in conventional arable cultivations and response to straw applications

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ABSTRACT

This work assessed populations of the anecic, deep burrowing earthworm *Lumbricus terrestris* on two recently established (3 years) and two long running (20–170 years) organic matter amended, conventionally managed arable field trials in SE England. Validated midden counts and DNA analyses were used to estimate *L. terrestris* populations and check species identity (>98% match, n = 10). Population estimates ranged between 0 and 1.3 *L. terrestris* middens per m^2 on conventionally (inorganic fertiliser only) managed plots. Surface wheat straw applications ($p \leq 0.05$) or wastes mixed with barley straw ($p \leq 0.05$) enhanced *L. terrestris* midden abundances. However, these were very low at <4.6 *L. terrestris* middens per m^2 and a population collapse was recorded under oat cropping. We found a residual population ranging between 0.1–3.6 *L. terrestris* middens per m^2 on the long running field trials. Further investigations are needed to identify if *L. terrestris* is functionally extinct at these densities.

1. Introduction

Anecic, deep burrowing earthworms play an important role in soil function; for example, *Lumbricus terrestris* is associated with soil pore formation and water infiltration (Edwards et al., 1990; Shipitalo and Butt, 1999) that supports crop productivity (Andriuzzi et al., 2015). Further, intensive tillage (ploughing) reduces anecic populations significantly (Chan, 2001) and long term intensive cultivations are linked to local extinctions (Kladivko et al., 1997). Ploughing has dominated (60–95%) UK arable cultivations over the past 30 years (Knight et al., 2012). Reduced tillage intensity is generally associated with higher densities of earthworms (Whalen and Fox, 2006). However, *L. terrestris* populations can remain virtually absent despite conversion to zero tillage (Crittenden et al., 2015). Field margins have high *L. terrestris* densities but do not act as a source of earthworms for field recolonization, indicating that the recovery of earthworm populations relies on the residual, surviving in-field worm populations (Roarty and Schmidt, 2013).

Organic matter amendments are one management strategy that could be used to improve earthworm populations e.g. (Leroy et al., 2008), however, few studies have investigated the role of in-field applications to specifically target anecic, deep burrowing *L. terrestris* population abundances. Farmyard manure applications are associated with elevated *L. terrestris* abundances in both ploughed (Edwards and Lofty, 1982b) and minimum tillage arable systems (Stroud et al., 2016). As *L. terrestris* earthworms are predominantly surface feeders, we hypothesised that crop residues (straw) would be critical to their abundance and that after autumn ploughing, the surface application of straw would increase their populations significantly. Further, that mixtures of straw and wastes (e.g. anaerobic digestate, farmyard manure or compost) would enhance their in-field populations above that of the wastes applied individually.

This study quantified the populations of *L. terrestris* on four conventionally managed arable field trials with a history of organic matter applications in SE England (Rothamsted experimental farm). These methods included midden counting validated using mustard extractions (Singh et al., 2015; Stroud et al., 2016) and DNA analyses to confirm species identification. Midden counting is the only non-destructive method to estimate *L. terrestris* populations at field scales e.g. (Rossi and Nuutinen, 2004) which is essential to agro-ecosystem research, and has been successfully

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used to study low (<5 per m^2) anecic earthworm abundances (Simonsen et al., 2010).

2. Materials and methods

2.1. Field trials

Three field experiments were located at Rothamsted Research Farm (51.82 N and 0.37 W) Harpenden, UK which has a temperate climate in the South of England. The soil is characterised as a flinty clay loam of the Batcombe series, with total organic C 1.6% and pH 6.99. One field experiment was at Woburn Research farm (52.02 N and 0.62 W), Woburn, UK. The soil at Woburn is a sandy loam, with 9% clay, 1% organic C, and pH 6.0. The field trials are conventionally managed with agrochemicals, straw is baled and removed (unless an experimental treatment) and every autumn the soils are intensively cultivated (3-furrow mouldboard plough to ca. 25 cm).

2.2. Straw application field trial

This trial is a complete randomised block design with four replicate plots per treatment and the crops receive 190 kg N ha^{-1} each year. The straw treatments have been annually applied to the plots for three years prior to *L. terrestris* sampling, as three years of amendments have been linked to population stabilisation (Leroy et al., 2008). The trial was in its third year of winter wheat (*Triticum aestivum* cv. Crusoe). The plot treatments selected for analysis were the control (no wheat straw), chopped straw (4.5 t ha^{-1}, ploughed in), regularly applied chopped straw (4.5 t ha^{-1}, applied as four amendments of 1.125 t ha^{-1}, with the first amendment ploughed in and subsequent amendments supplied to the soil surface over winter and spring), and a high rate of chopped straw (19 t ha^{-1}, which was ploughed in but the excess of straw (four times the normal rate) left residues on the surface). Earthworm surveys were performed on these 16 plots in April 2015 (Spring) and after harvest in September 2015 (Autumn) when *L. terrestris* are most active (Nieminen et al., 2015).

2.3. Straw and waste amendments field trial

This trial is a complete randomised block design, the treatments chosen for analysis were the control (no organic amendment) and anaerobic digestate, compost or farmyard manure, anaerobic digestate + barley straw, compost + barley straw, or farmyard manure + barley straw applied at 2.5 t C ha^{-1}. These organic matter treatments had been applied to the plots for three years prior to *L. terrestris* sampling. Four replicate plots per treatment were studied (28 plots) and the trial was cultivated with winter oats (*Avena sativa* cv. Gerald). Previous cropping in the rotation was spring barley *Hordeum vulgare* L. cv. *Tipple*), in 2014 and winter wheat (*Triticum aestivum* cv. Crusoe) in 2013. All plots receive annual inorganic N as recommended in RB209 (DEFRA, 2010) as appropriate for the rotation (120 kg N ha^{-1} for this oat crop). Earthworm surveys were performed in April 2015 (Spring) and after harvest in September 2015 (Autumn).

2.4. Broadbalk long-running field trial

The nearby long term experiment (ca. >170 years) Broadbalk has historical earthworm records (Edwards and Lofty, 1982b) and has been annually cultivated with winter wheat. We were able to study two plots for midden assays (5 m^2 per plot) with 1 m^2 mustard validation assays. The two plots were (i) 35 t farmyard manure plus 96 kg N ha^{-1}, and (ii) 144 kg N ha^{-1} only. Earthworm surveys were performed in September 2015, just prior to harvest.

2.5. Woburn long-running field trial

The long term experiment (20 years) at Woburn (Gibbs et al., 2006) was sampled which has historical earthworm records (Edwards and Lofty, 1982a), and has been annually cultivated with winter wheat. The trial is a complete randomised block design, with 8 replicates per treatment. The treatments selected for analysis were the controls (no biosolids addition), and uncontaminated biosolids additions (both annual and past treatments, 24 plots). Earthworm surveys were performed in September 2015, just prior to harvest.

2.6. L. terrestris *earthworm surveys*

A 1 m^2 square quadrat was used to transect ca. 20% of each plot area and the number of middens were recorded. The areas surveyed per plot were: straw application trial (10 m^2 per plot, 160 m^2 field surveyed), straw and waste amendments trial (12 m^2 per plot, 336 m^2 field surveyed), Broadbalk (5 m^2 per plot, 10 m^2 surveyed) and Woburn (14 m^2 per plot, 336 m^2 surveyed). Standard procedures were used to validate the midden counts (Singh et al., 2015; Stroud et al., 2016). Briefly, 1.5 l mustard extractions (a solution of 10 g mustard powder to 0.75 l water) were poured within a 0.25 m^2 square quadrat in a random location within the plot (midden densities often <1 in 0.25 m^2) to reflect the plot midden densities and earthworms were collected for analysis (species identity using the OPAL key) and released after assessment. Spearman's rank correlation co-efficient was used to assess the correlation to midden counts ($n = 57$, $R = 0.587$, $p < 0.001$) and the graph is shown in SI Fig. 1 To investigate the earthworms inhabiting middens, after a preceding days' rainfall event, middens were selected on the straw application field trial (12 plots, 10 middens per plot, $n = 120$) and 20 ml of mustard solution (as above) was syringed directly into the burrow and timed for 5-min to recover the inhabitant. *L. terrestris* was the only species recovered, the recovery rate was $50 \pm 7\%$ ($\pm$ standard error), with an average mass of 3.1 ± 0.3 g wet weight ($n = 57$, there were three escapees) and 12% of these recovered earthworms were adults.

2.7. L. terrestris *species identity using DNA*

To obtain a representative sample of *L. terrestris* specimens given their low abundance, large earthworms were collected during the ploughing of the straw field trial in September 2015. The *L. terrestris* earthworms selected for analysis ($n = 10$) were the most dissimilar (length, biomass and colour). They were killed with ethanol and immediately shipped for analysis by Eurofins Genomics. DNA was extracted using a commercial kit, PCR and primers were not successful following James et al. (2010), and so primers LepF1/LepR1 were used instead. Species identification was determined by sequence comparison with database entries in NCBI and BOLD.

2.8. Data analyses

Genstat (2012, 14th addition, VSN International Ltd., UK) was used to perform the statistical analyses. For the straw amendment trial, general ANOVA (Analysis of Variance) was used with the following parameters: Block = Block/Plot/Timing, Treatments = (straw/application)*timing; where 'straw' and 'application' were two factor categories respectively, comparing presence/absence of straw, and presence/absence of surface straw. The residual graphs were checked to meet the normality assumption (SI Fig. 2). For the straw and waste amendments trial, general ANOVA (Analysis of Variance) was used with the following parameters: Block = Block/Plot, Treatments = split/(mixture/organic matter type); where 'split' and 'mixture' were two factor categories comparing presence/absence of organic amendment and whether amendment was applied single waste or mixed with straw respectively. The residual graphs indicated that a transformation was required to meet the normality assumption (SI Fig. 3a), and a square root transformation was used (SI Fig. 3b). Only one time point was included (Spring) in the analysis because no middens were found in the Autumn. The Woburn long-running field trial was assessed by a general ANOVA, using the block and treatment parameters. The residual graphs indicated that a transformation was needed to meet the normality assumption (SI Fig. 4a) and again a square root transformation was used (SI Fig. 4b). Differences obtained at levels $p \leq 0.05$ were reported as significant.

3. Results and discussion

DNA analysis of the *L. terrestris* earthworms (determined using the OPAL key) confirmed that all specimens were *L. terrestris* (98–100% match), S1 Table 1 . These results are in agreement with a national survey of the cryptic diversity of earthworms including *L. terrestris* which identified no cryptic diversity of this species (King et al., 2008). Further, this suggests that standard earthworm keys on live specimens can be used to successfully monitor *L. terrestris* populations.

3.1. Straw application field trial

We hypothesised that straw, specifically the regular surface application of chopped straw would enhance the in-field populations of *L. terrestris*, as they are surface feeding earthworms and this would provide a source of food and midden building materials. The application of chopped wheat straw had a statistically significant effect on the abundance of *L. terrestris* ($F_{1,10} = 6.71$, $p = 0.03$), and the presence of surface wheat straw was also statistically significant ($F_{1,10} = 5.47$, $p = 0.04$) indicating that our hypothesis was correct (Fig. 1). Thus, autumn ploughing followed by the regular application of surface straw residues can enhance *L. terrestris* populations in-field, and this is most likely due to the provision of food and midden building materials at a time when they are most active (Nieminen et al., 2015). Spring cultivations where both the soil and residue are left undisturbed over the autumn and winter are linked to best supporting *L. terrestris* foraging and mating activities from their permanent burrow (Nuutinen, 1992). However, although *L. terrestris* abundance was stimulated by straw applications it is debatable whether these low abundances (1.18–4.64 *L. terrestris* middens per m^2, n = 24) would be sufficient for *L. terrestris* to contribute to ecosystem functioning.

The numbers of *L. terrestris* on the control plots (only receiving inorganic N) were 3–8 fold lower than the straw amended plots, with populations ranging between 0.15 per m^2–1.3 per m^2 middens during the year. A statistically significant ($F_{1,13} = 32.09$, $p < 0.001$) increase in the *L. terrestris* population was detected between spring and autumn samplings across this field (Fig. 1).

3.2. Straw and waste amendments field trial

We hypothesised that the application of organic amendments (compost, farmyard manure or anaerobic digestate) would enhance the abundance of *L. terrestris*, which was found ($F_{1,22} = 9.16$, $p = 0.006$). We also hypothesised that straw-waste mixtures, that is, adding straw to waste materials (compost, farmyard manure or anaerobic digestate) would enhance infield *L. terrestris* populations more than the wastes applied alone, which was found ($F_{1,22} = 4.31$, $p = 0.05$) (Fig. 2) in Spring, indicating that our hypothesis was correct. There was no significant difference in *L. terrestris* abundances between the amendment type (compost, farmyard manure or anaerobic digestate) ($F_{2,22} = 0.04$, $p > 0.05$), with population estimates <0.8 *L. terrestris* middens per m^2 for amendment treatments. However, these populations are extremely low, for example, the plots that only received inorganic N had a population estimate of 0.08 *L. terrestris* middens per m^2. The

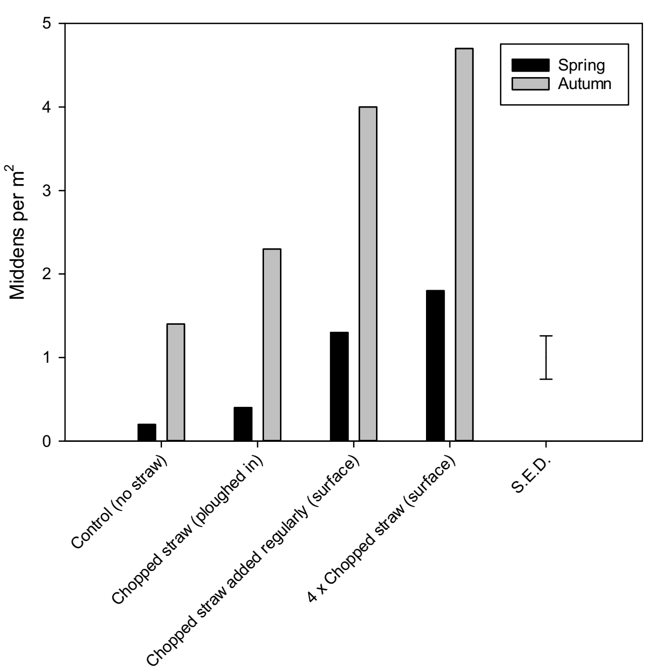

Fig. 1. Midden counts (per m^2) (±S.E.D. standard error of the differences) from the straw application field trial (n = 16, 4 plots per treatment) in spring and autumn 2015. The application of chopped straw and the presence of surface straw had a statistically significant effect on the abundance of *Lumbricus terrestris* ($p = 0.03$ and $p = 0.04$, respectively).

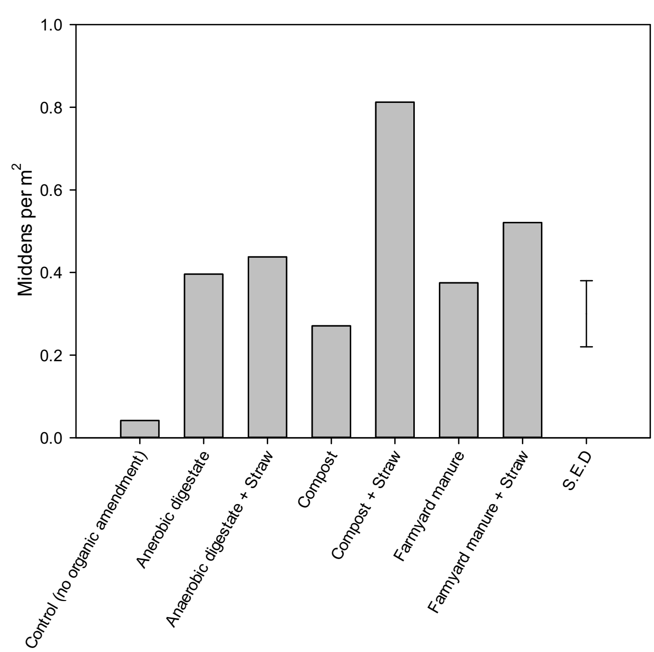

Fig. 2. Midden counts (per m^2) (±S.E.D. standard error of the differences) from the mixed straw and waste amendment field trial (n = 28, 4 plots per treatment) in spring only. In autumn zero *Lumbricus terrestris* middens were recorded. The application of all organic amendments and the mixed straw-waste treatments significantly enhanced abundance of *L.terrestris* ($p = 0.006$ and $p = 0.05$, respectively).

temporary improvement in their populations from the organic amendment additions was not detected in the autumn when zero *L. terrestris* middens were found, indicating a complete population collapse across this field. Oat cropping is implicated in this seasonal decline in *L. terrestris* populations as the opposite trend (improvement in midden numbers, Fig. 1) was found on the straw application field trial also at Rothamsted which was cultivated under winter wheat. Whilst we directly observed *L. terrestris* interact with wheat crop leaves (i.e. incorporation into their middens) and residues after harvest on the straw application trial, no oat leaf or harvest residue interactions were observed on the straw and waste amendments trial. It has been shown that *L. terrestris* avoids oat sown habitats and oat residues for food, associated with an allelopathic effects (Valckx et al., 2011). However, this is only based on two data points and further research is needed to understand the impact of crop rotation on the fluctuation of *L. terrestris* populations in arable systems as this could have wider implications to agro-ecosystem functioning.

3.3. Broadbalk long-running field trial

Autumn population estimates using midden counts generated population estimates of *L. terrestris* of 0.3 middens per m^2on plots amended with FYM, and on the inorganic amendment plot we recorded midden counts of 0.13 middens per m^2. No comparison can be made to historical data generated using formalin (which has been discontinued due to health risks) and modern techniques used for these assessments.

3.4. Woburn long-running field trial

Populations of *L. terrestris* in Woburn soils were recorded to decline by 73% from 29 per m^2 to 7.75 per m^2 by 1971 due to annual tillage (Edwards and Lofty, 1982a). Autumn 2015 *L. terrestris* midden abundances were 3 ± 1.2 per m^2 on non-biosolid amended plots and 3.6 ± 0.5 per m^2 on biosolids amended plots.These data indicate that biosolids are not a useful amendment to stimulate *L. terrestris* earthworm populations under these tillage management practices, as there was no significant ($F_{7,14} = 0.31$, $p > 0.05$) difference between *L. terrestris* populations on the control plots and biosolid amended plots. Populations of anecic earthworms are estimated to be <3 per m^2 on ploughed systems (Simonsen et al., 2010), in agreement with our findings of *L. terrestris* residual populations across these field sites.

4. Conclusion

There is a small residual population of *L. terrestris* earthworms in arable, intensively cultivated soils ranging between 0.08–3.6 middens per m^2. Their populations are enhanced by organic matter applications, with the most noticeable response to wheat straw. However, these populations never exceeded 4.6 per m^2 and collapsed under oat cropping. Further investigations are needed to identify if *L. terrestris* is functionally extinct in conventionally managed arable ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.08.002.

References

Andriuzzi, W.S., Pulleman, M.M., Schmidt, O., Faber, J.H., Brussaard, L., 2015. Anecic earthworms (*Lumbricus terrestris*) alleviate negative effects of extreme rainfall events on soil and plants in field mesocosms. Plant Soil 397, 103–113.

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity – implications for functioning in soils. Soil Tillage Res. 57, 179–191.

Crittenden, S.J., Huerta, E., de Goede, R.G.M., Pulleman, M.M., 2015. Earthworm assemblages as affected by field margin strips and tillage intensity: an on-farm approach. Eur. J. Soil Biol. 66, 49–56.

DEFRA, 2010. Fertiliser Manual (RB209), 8th ed. TSO, Great Britain.

Edwards, C.A., Lofty, J.R., 1982a. The effect of direct drilling and minimal cultivation on earthworm populations. J. Appl. Ecol. 19, 723–734.

Edwards, C.A., Lofty, J.R., 1982b. Nitrogenous fertilizers and earthworm populations in agricultural soils. Soil Biol. Biochem. 14, 515–521.

Edwards, W.M., Shipitalo, M.J., Owens, L.B., Norton, L.D., 1990. Effect of *Lumbricus terrestris* L. burrows on hydrology of continuous no-till corn fields. Geoderma 46, 73–84.

Gibbs, P.A., Chambers, B.J., Chaudri, A.M., McGrath, S.P., Carlton-Smith, C.H., Bacon, J. R., Campbell, C.D., Aitken, M.N., 2006. Initial results from a long-term, multi-site field study of the effects on soil fertility and microbial activity of sludge cakes containing heavy metals. Soil Use Manage. 22, 11–21.

James, S.W., Porco, D., Decaëns, T., Richard, B., Rougerie, R., Erséus, C., 2010. DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): resurrection of *L. herculeus* (Savigny, 1826). PLoS One e15629.

King, R.A., Tibble, A.L., Symondson, W.O., 2008. Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Mol. Ecol. 17, 4684–4698.

Kladivko, E.J., Akhouri, N.M., Weesies, G., 1997. Earthworm populations and species distributions under no-till and conventional tillage in Indiana and Illinois. Soil Biol. Biochem. 29, 613–615.

Knight, S., Knightley, S., Bingham, I., Hoad, S., Lang, B., Philpott, H., Stobart, R., Thomas, J., Barnes, A., Ball, B., 2012. Desk study to evaluate contributory causes of the current yield plateau in wheat and oilseed rape. HGCA Report No 502. Home Grown Cereals Authority, Stoneleigh, Warwickshire.

Leroy, B.L.M., Schmidt, O., Van den Bossche, A., Reheul, D., Moens, M., 2008. Earthworm population dynamics as influenced by the quality of exogenous organic matter. Pedobiologia 52, 139–150.

Nieminen, M., Hurme, T., Mikola, J., Regina, K., Nuutinen, V., 2015. Impact of earthworm *Lumbricus terrestris* living sites on the greenhouse gas balance of no-till arable soil. Biogeosciences 12, 5481–5493.

Nuutinen, V., 1992. Earthworm community response to tillage and residue management on different soil types in southern Finland. Soil Tillage Res. 23, 221–239.

Roarty, S., Schmidt, O., 2013. Permanent and new arable field margins support large earthworm communities but do not increase in-field populations. Agric. Ecosyst. Environ. 170, 45–55.

Rossi, J.P., Nuutinen, V., 2004. The effect of sampling unit size on the perception of the spatial pattern of earthworm (*Lumbricus terrestris* L.) middens. Appl. Soil Ecol. 27, 189–196.

Shipitalo, M.J., Butt, K.R., 1999. Occupancy and geometrical properties of *Lumbricus terrestris* L-burrows affecting infiltration. Pedobiologia 43, 782–794.

Simonsen, J., Posner, J., Rosemeyer, M., Baldock, J., 2010. Endogeic and anecic earthworm abundance in six Midwestern cropping systems. Appl. Soil Ecol. 44, 147–155.

Singh, P., Heikkinen, J., Ketoja, E., Nuutinen, V., Palojärvi, A., Sheehy, J., Esala, M., Mitra, S., Alakukku, L., Regina, K., 2015. Tillage and crop residue management methods had minor effects on the stock and stabilization of topsoil carbon in a 30-year field experiment. Sci. Total Environ. 518–519, 337–344.

Stroud, J.L., Irons, D., Watts, C.W., Whitmore, A.P., 2016. *Lumbricus terrestris* abundance is not enhanced after three years of compost amendments on a reduced tillage wheat cultivation conversion. Appl. Soil Ecol. 98, 282–284.

Valckx, J., Pina, A.C., Govers, G., Hermy, M., Muys, B., 2011. Food and habitat preferences of the earthworm *Lumbricus terrestris* L. for cover crops. Pedobiologia 54 (Supplement), S139–S144.

Whalen, J., Fox, C.A., 2006. Biodiversity of lumbricid earthworms in temperate agroeco-systems. In: Benckiser, G., Schnell, S. (Eds.), Biodiversity In Agricultural Production Systems. CRC Press p. 429.